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Date: October 3, 1997

Docket No.: 2185-0208P-SP

Assistant Commissioner for Patents
Box PATENT APPLICATION
Washington, D.C. 20231

Sir:

As authorized by the inventor(s), transmitted herewith for filing
is a patent application applied for on behalf of the inventor(s)
according to the provisions of 37 CFR 1.41(c).

Inventor(s): KOSHIBA, Tomokazu

For: ALDEHYDE OXIDASE GENE DERIVED FROM PLANT AND UTILIZATION
THEREOF

Enclosed are:

- X A specification consisting of 58 pages
- sheet(s) of drawings
- Certified copy of Priority Document(s)
- X Executed Declaration in accordance with 37 CFR 1.64 will follow
- A verified statement to establish small entity status under 37
CFR 1.9 and 37 CFR 1.27
- X Preliminary Amendment
- X Information Sheet
- Information Disclosure Statement, PTO-1449 with reference(s)

Other _____

The filing fee has been calculated as shown below:

			LARGE ENTITY		SMALL ENTITY	
FOR	NO. FILED	NO. EXTRA	RATE	FEE		RATE FEE
BASIC FEE	*****	*****	*****			****
	*****	*****	*****	\$790.00	or	**** \$395.00
	*****	*****	*****			****
TOTAL CLAIMS	58 - 20 =	38	x22 = \$	836.00	or	x 11 = \$ 0.00
INDEPENDENT	2 - 3 =	0	x82 = \$	0.00	or	x 41 = \$ 0.00
MULTIPLE DEPENDENT CLAIM PRESENTED <u>yes</u>				+270 = \$270.00	or	+135 = \$ 0.00
			TOTAL \$1,896.00		TOTAL \$ 0.00	

X The application transmitted herewith is filed in accordance with 37 CFR 1.41(c). The undersigned has been authorized by the inventor(s) to file the present application. The original duly executed patent application together with the surcharge will be forwarded in due course.

X A check in the amount of \$1,896.00 to cover the filing fee and recording fee (if applicable) is enclosed.

_____ The Government Filing Fee will be paid at the time of completion of the filing requirement.

_____ Please charge Deposit Account No. 02-2448 in the amount of \$_____. A triplicate copy of this transmittal form is enclosed.

X Send Correspondence to: BIRCH, STEWART, KOLASCH & BIRCH, LLP
P. O. Box 747
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X If necessary, the Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 02-2448.

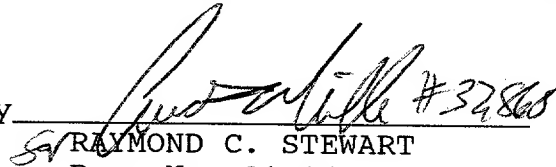
X Any additional filing fees required under 37 CFR 1.16.

X Any patent application processing fees under 37 CFR 1.17.

Respectfully submitted,

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By

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: T. KOSHIBA
Serial No.: NEW Group:
Filed: October 3, 1997 Examiner:
For: ALDEHYDE OXIDASE GENE DERIVED FROM PLANT AND
UTILIZATION THEREOF

PRELIMINARY AMENDMENT

Honorable Commissioner of Patents
and Trademarks
Washington, D.C. 20231

October 3, 1997

Sir:

Applicants respectfully submit the following amendments and
remarks in connection with the above-identified new application:

IN THE CLAIMS:

Claim 3, lines 1-2, delete "or 2".

REMARKS

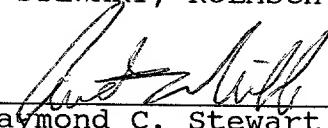
It is submitted that the above amendment is made merely to
delete the improper multiple dependency of the claim and does not
constitute new matter. Favorable action on the above-identified
application is respectfully requested.

Please charge any fees or credit any overpayment pursuant to
37 CFR 1.16 or 1.17 to Deposit Account No. 02-2448.

Respectfully submitted,

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IN THE U.S. PATENT AND TRADEMARK OFFICE

I N F O R M A T I O N S H E E T

Applicant: KOSHIBA, Tomokazu

Serial No.:

Filed: October 3, 1997

For: ALDEHYDE OXIDASE GENE DERIVED FROM PLANT AND UTILIZATION
THEREOF

Priority Claimed:

COUNTRY	DATE	NUMBER
Japan	10/04/96	08-283314

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The above information is submitted to advise the USPTO of all relevant facts in connection with the present application. A timely executed Declaration in accordance with 37 CFR 1.64 will follow.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

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ALDEHYDE OXIDASE GENE DERIVED FROM PLANT AND UTILIZATION
THEREOF

FIELD OF THE INVENTION

The present invention relates to an aldehyde oxidase
5 gene derived from a plant and utilization thereof.

It has been known that a natural plant growth hormone
auxin alternatively IAA or indoleacetic acid is produced from
tryptophane via indoleacetaldehyde followed by the action
10 of an oxidase in higher plants. The hormone is deeply involved
in various morphogenesis and environmental adaptation of a
plant by its physiological activity and has significant
effects on maturing by growth acceleration in general crop
cultivation, improvement in yield and in quality by rooting
15 acceleration in nursery plant production, increase in yield
by growth acceleration of fruits in fruit vegetable
cultivation, increase in added value by acceleration of
flowering and elongation of life by prevention of defoliation
or aging in ornamental plant cultivation. Therefore, there
20 has been a strong demand for a method for artificially
controlling the said enzyme for industry and particularly
agricultural production.

Under these circumstances, the present inventors have
successfully determined the total amino acid sequence and
25 gene of the enzyme and completed the present invention.

Thus, the present invention provides:

- 1) An aldehyde oxidase gene which is a 4.4 Kbp gene obtainable from a plant and which encodes an amino acid sequence of an enzyme capable of oxidizing an aldehyde compound to a carboxylic acid (hereinafter, referred to as the gene of the present invention),
- 2) The aldehyde oxidase gene according to item 1), wherein the aldehyde compound is indoleacetaldehyde and the carboxylic acid is indoleacetic acid,
- 3) The aldehyde oxidase gene according to item 1 or 2 which is derived from a maize plant (*Zea mays L.*),
- 4) The aldehyde oxidase gene according to item 1 which is a nucleotide sequence encoding an amino acid sequence shown by SEQ ID NO: 1,
- 5) The aldehyde oxidase gene according to item 4 which has a nucleotide sequence shown by SEQ ID NO: 2 (loci of CDS being 46..4120),
- 6) The aldehyde oxidase gene according to item 1 which is a nucleotide sequence encoding an amino acid sequence shown by SEQ ID NO: 3,
- 7) The aldehyde oxidase gene according to item 6 which has a nucleotide sequence shown by SEQ ID NO: 4 (loci of CDS being 91..4138),
- 8) A plasmid comprising the aldehyde oxidase gene according to item 1, 2, 3, 4, 5, 6 or 7,

- 9) A transformant transformed by introducing the plasmid according to item 8 into a host cell,
- 10) The transformant according to item 9, wherein the host cell is a microorganism,
- 5 11) The transformant according to item 9, wherein the host cell is a plant,
- 12) A process for constructing an expression plasmid which comprises ligating:
- (1) a promoter capable of functioning in a plant cell,
- 10 (2) an aldehyde oxidase gene according to item 1, 2, 3, 4, 5, 6 or 7 and
- (3) a terminator capable of functioning in a plant in a functional manner and in the said order described above,
- 13) An expression plasmid comprising:
- 15 (1) a promoter capable of functioning in a plant cell,
- (2) an aldehyde oxidase gene according to item 1, 2, 3, 4, 5, 6 or 7 and
- (3) a terminator capable of functioning in a plant which are ligated in a functional manner and in the said order
- 20 described above,
- 14) A process for controlling production of an aldehyde oxidase in a transformant which comprises introducing, into a host cell, an expression plasmid comprising:
- 25 (1) a promoter capable of functioning in a plant cell,

(2) an aldehyde oxidase gene and
(3) a terminator capable of functioning in a plant which are
ligated in a functional manner and in the said order
described above to transform said host cell,

5 15) The process according to item 14, wherein the
aldehyde oxidase gene is derived from a plant and the host
cell is a plant, and

 16) The process according to item 14, wherein the
expression plasmid is the expression plasmid according to
10 item 13.

EMBODIMENTS OF THE INVENTION

The present invention will be described in more detail.

The gene of the present invention comprises about 4.4
kbp nucleotide obtainable from a plant and is an aldehyde
15 oxidase gene that encodes an amino acid sequence of an enzyme
capable of oxidizing an aldehyde compound to generate a
carboxylic acid. For example, it is capable of oxidizing
indoleacetaldehyde to generate indoleacetic acid.

The gene of the present invention can be obtained from
20 a plant, for example, maize or the like. The gene of the
present invention and the enzyme as the translation product
of it have an action of oxidizing an acetaldehyde compound
to a carboxylic acid in a cell. Said enzyme may also act,
for example, on benzaldehyde, butyraldehyde,
25 protocatechualdehyde or the like as the substrate, in

addition to indolealdehyde. Of course, a single enzyme may act on plural compounds as substrates.

The gene of the present invention specifically includes,
5 for example, a gene which is a nucleotide sequence encoding an amino acid sequence shown by SEQ ID NO: 1 and a gene which is a nucleotide sequence encoding an amino acid sequence shown by SEQ ID NO: 3 as well as an equivalent of them. The expression "an equivalent of them" used herein means an
10 aldehyde oxidase gene having a nucleotide sequence of an aldehyde oxidase gene that encodes an amino acid sequence shown by SEQ ID NO: 1 or SEQ ID NO: 3 with a single nucleotide or plural nucleotides added, deleted or replaced, and refers to a DNA which is an analog having the same function. More
15 particularly, this includes a gene having a nucleotide sequence shown by SEQ ID NO: 2 (loci of CDS being 46..4120) or a nucleotide sequence shown by SEQ ID NO: 4 (loci of CDS being 91..4138).

The gene of the present invention can be obtained by
20 the following process.

For example, seeds of Golden Cross Bantam 70 (purchased from Sakata-no-tane), a maize cultivar, are subjected to a treatment for hastening of germination by immersing
overnight in running tap water, subsequently seeded on a paper
25 towel moistened with water and placed in red light (0.8 W/m^2)

under a condition of 25°C for 2 days and then in the dark for 1 day to allow germination. Top portions of young sheaths grown to 1.0 - 1.5 cm from the obtained seedlings are excised under a green safety light, immediately frozen with liquid nitrogen and stored at -30°C as samples for purification of enzymes and samples for extracting RNAs.

For purifying aldehyde oxidase from the frozen samples prepared in this manner, it is appropriate to use a method described in T. Koshiba et al., Plant Physiology, 1996, 110, 781 - 789.

In order to prevent decrease in activity of the enzyme and decomposition of the protein during procedures of extraction and purification, it is preferred to carry out all the treatments in the purification steps at a lower temperature of 2 - 4°C, as is ordinary manner in such procedures. First, 150 - 200 g of the frozen sample is taken as a material for one batch of purification. The material is mechanically crushed by a homogenizer or the like with addition of 400 ml of 0.1 M phosphate buffer (pH 7.4) and centrifuged at 12,000 g for 30 minutes. The supernatant is separated as a crude enzyme standard sample. From the crude enzyme standard sample, a fraction is obtained with 30 - 50% saturated ammonium sulfate, dialyzed against 20 mM Tris HCl buffer (pH 8.0) and centrifuged at 20,000 g for 20 minutes. The supernatant from centrifugation is passed over an

ion-exchange column (for example, DEAE TOYOPEARL 650 M, manufactured by Tosoh) and a fraction with an aldehyde oxidase activity is collected. Said fraction with the specific activity is subjected to chromatography with a hydrophobic column, a hydroxyapatite column and an ion-exchange column (for example, DEAE-5PM) in this order and purified until the fraction with aldehyde oxidase activity is detected as an almost single protein band by silver staining after electrophoresis.

10 According to the above described purification procedure, about 2,000 times purification, in terms of the amount of protein in the crude enzyme standard sample, is usually possible. It can be confirmed that the finally purified protein has a size of about 300 kD in molecular weight by the gel filtration column process. Further, it can be
15 detected as a band having a size of about 150 kD in molecular weight by SDS polyacrylamide gel electrophoresis (SDS-PAGE), indicating that said enzyme forms a dimer.

In the above described fractionating process by column
20 chromatography, effective collection of the fraction with aldehyde oxidase activity can be achieved making use of measurement of aldehyde oxidase activity in respective fractions. For this purpose, a method in which indoleacetaldehyde is added to the purified fraction as a
25 substrate and the amount of produced indoleacetic acid is

determined by HPLC, for example, can be utilized. Precisely, 100 μ l of reaction solution consisting of 5 - 50 μ l of the purified fraction, 0.1 mM indoleacetaldehyde and 0.1 mM phosphate buffer (pH 7.4) is prepared. The solution is
5 incubated at 30°C for 30 minutes to effect the reaction and, immediately after, 8 μ l of 1 N HCl, 5 μ l of 2.0 M sodium hydrogen sulfite and 50 μ l of methanol are added to the solution to quench the reaction. The reaction solution is centrifuged at 15,000 g for 5 minutes and 100 μ l of the
10 obtained supernatant is taken as a analytical sample for HPLC.

By detecting absorption at 280 nm, indoleacetaldehyde as the substrate and indoleacetic acid as the reaction product can be quantitatively analyzed. It is effective to carry out HPLC with, for example, ODS C18 column and to elute with 20
15 - 50% linear gradient of methanol containing 0.1% acetic acid.

The protein obtained in this manner is partially digested and the digested peptide is analyzed to obtain a partial amino acid sequence information. Usually, the purified aldehyde oxidase sample is separated by SDS-PAGE
20 and a protein band of 150 kD is collected by excision. The collected gel fragments are treated, for example, with Achromobacter Protease I (API) in the presence of 0.1% SDS and digested peptide fragments are extracted. This is loaded, for example, on a reverse phase HPLC accompanied by a
25 pre-column of an anion exchanger (DEAE) to separate peptides

and recover them. The amino acid sequences are determined by a protein sequencer and parts of the samples are subjected to molecular weight determination by MALDI-TOF to check accuracy of the obtained amino acid sequence information.

5 Then, an oligo DNA expected to encode the amino acid sequence is synthesized on the basis of the obtained amino acid sequence information. Further, RT-PCR is conducted using a total RNA as a template to amplify cDNA partial fragment, which is then cloned into a plasmid vector.

10 For extraction of the total RNA, 7 g of the frozen sample, for example, is triturated in liquid nitrogen with a mortar and a pestle to form fine powders. After evaporating liquid nitrogen, RNA is extracted by the conventional manner, for example, using guanidine thiocyanate/cesium chloride
15 process and the total RNA is collected from the extract by ethanol precipitation. By this procedure, usually 1 mg of the total RNA is obtained.

For amplification of cDNA, a reverse transcription reaction is carried out using, among synthetic oligo DNAs,
20 one synthesized in antisense orientation as a primer and binding it to a transcription product of a target RNA contained in the total RNA. The reverse transcription reaction can be conducted using a commercially available reverse transcription PCR kit, for example, RNA-PCR kit
25 (manufactured by Perkin-Elmer Cetus Instruments). Then, the

obtained reverse transcription product can be subjected again to PCR in which an oligo DNA synthesized in sense orientation is added to amplify cDNA fragment.

The obtained cDNA amplification fragment is purified
5 and cloned into a plasmid vector. As the plasmid vector, for example, pCRII (manufactured by Invitrogen) can be used and cDNA amplification fragment can be cloned by transforming *E. coli* according to the conventional manner and screening transformants having an insert. The nucleotide sequence of
10 the clone is determined using, for example, ABI PRISM Dye Primer Cycle Sequencing Ready Reaction Kits (manufactured by Applied Biosystems) on the obtained cDNA clone.

Sense and antisense primers for part of nucleotide sequence in cDNA partial fragment obtained in this manner
15 can be synthesized and subjected to RACE to obtain cDNA fragments having terminals in 5'-orientation and 3'-orientation, respectively. A complete length cDNA can be obtained by ligating them and cloning into a plasmid vector.

For the RACE, a commercially available Marathon cDNA
20 Amplification Kit (manufactured by Clontech), for example, can be used.

The gene of the present invention can be utilized in the following manner.

For example, a host cell such as a microorganism, a plant
25 or the like is transformed by introducing the gene of the

present invention to form a transformant.

In order to introduce and express the gene of the present invention in a plant cell, an expression plasmid comprising (1) a promoter capable of functioning in a plant cell, (2) a gene of the present invention (an aldehyde oxidase gene described in items 1 to 7 above) and (3) a terminator capable of functioning in a plant cell which are ligated in a functional manner in a plant cell and in the said order described above and introduced in a plant cell to transform said cell.

The expression "in a functional manner" used herein means that, when the constructed plasmid is introduced into a plant cell to transform it, the gene of the present invention is integrated under the control of a promoter such that the gene is normally transcribed/translated and have a function of expressing a protein in said plant cell.

The promoter capable of functioning in a plant cell includes, for example, T-DNA derived constitutive type promoters such as nopaline synthase gene (NOS) promoter, octopine synthase gene (OCS) promoter and the like, plant virus derived promoters such as cauliflower mosaic virus (CaMV) derived 18S and 35S promoters and the like, and inducible type promoters such as phenylalanine ammonia-lyase (PAL) gene promoter, chalcone synthase (CHS) gene promoter, pathogen-related (PR) gene promoter and the like. Further,

it includes other known plant promoters.

The terminator capable of functioning in a plant cell includes, for example, T-DNA derived constitutive type terminators such as nopaline synthase gene (NOS) terminator
5 and the like, plant virus derived terminators such as garlic virus GV1, GV2 terminators and the like. Further, it includes other known plant terminators.

For transforming a plant cell by introducing such plasmid into a plant cell, the above described expression
10 plasmid is introduced into a plant cell by any of conventional means such as *Agrobacterium* infection method (JP-B-2-58917 and JP-A-60-70080), electroporation method into protoplast (JP-A-60-251887 and JP-A-5-68575), particle gun method (JP-A-508316 and JP-A-63-258525) and the like, and a
15 transformed plant cell can be obtained by selecting a plant cell into which the gene of the present invention is introduced. The transformed plant is obtained by regenerating a plant according to a conventional plant cell culturing process, for example, described in Uchimiya,
20 Manual for Plant Gene Manipulation (Method for Producing Transgenic Plants), Published by Kodansha Scientific (ISBN 4-06-153515-7 C3045), 1990, pages 27 - 55.

Further, the present invention provides a process for controlling production of an aldehyde oxidase in a
25 transformant which comprises introducing, into a host cell,

an expression plasmid comprising (1) a promoter capable of functioning in a plant cell, (2) an aldehyde oxidase gene and (3) a terminator capable of functioning in a plant which are ligated in a functional manner and in the said order
5 described above to transform said host cell.

The promoter capable of functioning in a plant cell includes, for example, lacZ gene promoter of lactose operon in *E. coli*, alcohol dehydrogenase gene (ADH) promoter in yeast, Adenovirus major late (Ad.ML) promoter, early promoter of
10 SV 40, Baculovirus promoter and the like. When the host is a plant, promoters capable of functioning in a plant as described above may also be included.

The terminator capable of functioning in a plant cell includes, for example, HIS terminator sequence in yeast, ADHI
15 terminator, early splicing region of SV 40 and the like. When the host is a plant, terminators capable of functioning in a plant as described above may also be included.

The aldehyde oxidase gene may be any one insofar as it is a gene encoding an amino acid sequence of an enzyme capable
20 of oxidizing an aldehyde compound to form a carboxylic acid.

This includes, for example, aldehyde oxidase genes derived from plants and preferably the gene of the present invention (an aldehyde oxidase gene described in items 1 to 7 above).

Transformation of a host cell by introducing such
25 plasmid into said host cell can be effected by a method

generally used in the field of genetic engineering.

When the host cell is a plant cell, it can be effected, for example, by a method generally used in the field of plant genetic engineering and the field of plant tissue cultivation
5 as described above.

The transformation of a plant by introducing the gene of the present invention may bring about enhancement of generally known physiological action of auxin or suppression of the same. For example, by enhancing the activity of auxin
10 through a sense gene, elongation growth and differentiation to vascular bundle of the host cell can be accelerated resulting in growth acceleration of a plant and enhanced capacity of storing assimilation products. As a result, early maturing of crops, enlargement of harvest such as fruits
15 and improvement in yield or quality can be expected and realized. To the contrary, by suppressing the activity of auxin through a sense gene, spindly growth of a plant is prevented and a plant capable of growing under improper environmental conditions such as insufficient insolation can
20 be bred. Further, by adequately controlling growth, dwarfing of crops becomes possible and application, for example, to prevention of lodging of rice plants and shortening of cut flowers become possible. As a result, improvement in yield and quality can be expected.

25 Addition of hormone to the medium is generally essential

for aseptic cultivation of cells or tissue of a plant. When auxin activity in a plant is enhanced by introducing and expressing the gene of the present invention thereby increasing production of aldehyde oxidase in a transformant, 5 said plant is expected to be in a state in which capacity of cell proliferation, differentiation and individual regeneration in the sterile culture is enhanced. Therefore, it is possible to create a so-called easily cultured strain and this is useful in the production of nursery plant of 10 virus-free crops for which tissue culture-nucleotide mass culture is conducted and garden crops such as flower and ornamental plants.

EXAMPLES

15 The present invention will now be described in more detail by means of Examples. It is to be understood, however, that the scope of the present invention is not limited to these Examples.

20 Example 1 (Preparation of maize young sheath)

Seeds of Golden Cross Bantam 70 (purchased from Sakata-no-tane), a maize cultivar, were subjected to a treatment for hastening of germination by immersing overnight in running tap water, subsequently seeded on a paper 25 towel moistened with water and placed in red light (0.8 W/m^2)

under a condition of 25°C for 2 days and then in the dark for 1 day to allow germination. Top portions (1.0 - 1.5 cm) of young sheaths grown from the obtained seedlings to 2 - 3 cm were excised under a green safety light, immediately frozen
5 with liquid nitrogen and stored at -30°C.

Example 2 (Preparation of aldehyde oxidase)

All the procedures in the following purification steps were conducted at a low temperature of 2 - 4°C.

10 First, about 200 g of the frozen sample prepared in Example 1 was taken as a material for one batch of purification.

The material was mechanically crushed by a homogenizer with addition of 400 ml of 0.1 M phosphate buffer (pH 7.4) and centrifuged at 12,000 g for 30 minutes. The supernatant was
15 separated as a crude enzyme standard sample. Subsequently, from the crude enzyme standard sample, a fraction was obtained with 30 - 50% saturated ammonium sulfate, dialyzed against 20 mM Tris HCl buffer (pH 8.0) and centrifuged at 20,000 g for 20 minutes. The supernatant from centrifugation was
20 passed over an ion-exchange column (DEAE TOYOPEARL 650 M, manufactured by Tosoh) and a fraction with an aldehyde oxidase activity was collected on the basis of activity measurement conducted in a manner described below in Example 3. Said fraction with activity was subjected to chromatography with
25 a hydrophobic column, a hydroxyapatite column and an

ion-exchange column (DEAE-5PM) in this order and purified until the fraction with aldehyde oxidase activity was detected as an almost single protein band by silver staining on electrophoresis.

5 By the above described purification procedure, about 0.09 mg of protein was recovered from 1,873 mg of protein in the crude enzyme standard sample, and ratio of enzyme activity for aldehyde oxidase to the original was 1,950 times.

10 It was confirmed that the finally purified protein had a size of about 300 kD in molecular weight by the gel filtration column process. Further, it was detected as a band having a size of about 150 kD in molecular weight by SDS polyacrylamide gel electrophoresis (SDS-PAGE), indicating that said enzyme formed a dimer.

15

Example 3 (Method for measuring aldehyde oxidase activity)

Measurement of aldehyde oxidase activity in the respective purified fractions described in Example 2 was carried out by a method in which indoleacetaldehyde was added to the purified fraction as a substrate and the amount of produced indoleacetic acid (IAA) was determined by HPLC. The reaction was carried out with 100 μ l of reaction solution consisting of 5 - 50 μ l of the purified fraction, 0.1 mM indoleacetaldehyde and 0.1 mM phosphate buffer (pH 7.4). The solution was incubated at 30°C for 30 minutes and, immediately

20

25

after, 8 μ l of 1 N HCl, 5 μ l of 2.0 M sodium hydrogen sulfite and 50 μ l of methanol were added to the solution to quench the reaction. The reaction solution was centrifuged at 15,000 g for 5 minutes and 100 μ l of the obtained supernatant was taken as a analytical sample for HPLC. By detecting absorption at 280 nm, indoleacetaldehyde and indoleacetic acid were quantitatively analyzed. HPLC was carried out with ODS C18 column and eluted with 20 - 50% linear gradient of methanol containing 0.1% acetic acid.

10

Example 4 (Peptide digestion of aldehyde oxidase: partial amino acid sequence)

The purified protein obtained in Example 2 was separated by SDS-PAGE and a protein band of 150 kD was collected by excision. The collected gel fragments were reacted with Achromobacter Protease I (API) in the presence of 0.1% SDS and digested peptide fragments were extracted. This was passed over a reverse phase HPLC accompanied by a pre-column of an anion exchanger (DEAE) to separate peptides, which were collected. The amino acid sequences were determined by a protein sequencer (ABI 477A).

15
20

As a result, the following 4 sequences were obtained as the partial amino acid sequences.

The first one was a sequence, shown below, having 18 amino acid residues:

25

Gln Val Asn Asp Val Pro Ile Ala Ala Ser Gly Asp Gly Trp Tyr His Pro Lys
and it was confirmed that the sequence corresponded to Nos.
235 to 252 residues in the amino acid sequence shown by SEQ
ID NO: 1.

5 The second one was a sequence, shown below, having 16
amino acid residues:

Thr Asn Ser Asp Gly Leu Val Ile His Asp Gly Thr Trp Thr Tyr Lys
and it was confirmed that the sequence corresponded to 1,234
to 1,249 residues in the amino acid sequence shown by SEQ
10 ID NO: 1 or to 1,226 to 1,241 residues in the amino acid
sequence shown by SEQ ID NO: 3.

 The third one was a sequence, shown below, having 20
amino acid residues:

Ser Ile Glu Glu Leu His Arg Leu Phe Asp Ser Ser Trp Phe Asp Asp Ser Ser
15 Val Lys
and it was confirmed that the sequence corresponded to Nos.
253 to 272 residues in the amino acid sequence shown by SEQ
ID NO: 1.

 The fourth one was a sequence, shown below, having 21
20 amino acid residues:

Val Gly Ala Glu Ile Gln Ala Ser Gly Glu Ala Val Tyr Val Asp Asp Ile Pro
Ala Pro Lys
and it was confirmed that the sequence corresponded to Nos.
591 to 611 residues in the amino acid sequence shown by SEQ
25 ID NO: 1.

Parts of these digested peptide samples were subjected to molecular weight determination by MALDI-TOF to check accuracy of the obtained amino acid sequence.

5 Example 5 (Preparation of total RNA from maize young sheath and synthesis of cDNA)

In a manner similar to that in Example 1, seeds of maize were germinated and 7 g of top portions of the young sheath were collected from seedlings. These were frozen in 10 ml of liquid nitrogen and triturated with a mortar and a pestle to form fine powders. After evaporating liquid nitrogen, RNA was extracted by the conventional manner (guanidine thiocyanate/cesium chloride method) and 1 mg of the total RNA was collected from the extract by ethanol precipitation.

15

Example 6 (Preparation of an oligo DNA primer and RT-PCR)

A mixture of oligo DNAs expected to encode the partial amino acid sequence determined in Example 4 was synthesized in both sense and antisense orientation.

20 Specifically, as a nucleotide sequence expected from 8 amino acid residues: Val Ile His Asp Gly Thr Trp Thr in the partial amino acid sequence 2 described in Example 4, a 23-mer in antisense orientation: 5'-

GTCCAIGTICC(AG)TC(AG)TGIATAC-3' was synthesized.

25 Further, as a nucleotide sequence expected from 8 amino

acid residues: Gly Glu Ala Val Tyr Val Asp Asp in the partial amino acid sequence 4 described in Example 4, a 23-mer in sense orientation: 5'-GGIGA(AG)GCIGTITA(TC)GTIGA(TC)GA-3' was synthesized.

5 A reverse transcription reaction was carried out using, among them, one synthesized in antisense orientation as a primer and a commercially available reverse transcription PCR kit (RNA-PCR kit, manufactured by Perkin-Elmer Cetus Instruments). Then, the obtained reverse transcription
10 product was subjected again to PCR in which an oligo DNA synthesized in sense orientation was added. As the result, amplification of cDNA fragment was confirmed.

Example 7 (Cloning of the PCR-amplified fragment into a vector
15 and analysis of the structure)

The amplified cDNA fragment obtained in Example 6 was purified and cloned into a plasmid vector pCRII (manufactured by Invitrogen). Further, the nucleotide sequence of the insert in said plasmid vector was determined by 373A DNA
20 Sequencer (manufactured by Applied Biosystems) using ABI PRISM Dye Primer Cycle Sequencing Ready Reaction Kits (manufactured by Applied Biosystems) and the structure of said cDNA fragment was determined. As a result, it was revealed that the cloned cDNA fragment contained 2 kinds
25 having different structure, one corresponding to Nos. 1,839

to 3,785 nucleotides in the nucleotide sequence shown by SEQ ID NO: 2 and the other corresponding to Nos. 1,858 to 3,806 nucleotides in the nucleotide sequence shown by SEQ ID NO: 4.

5

Example 8 (Isolation of a complete length cDNA clone)

Based on the nucleotide sequence information obtained in Example 7, nucleotide sequences specific for said 2 cDNAs, respectively, were searched and oligo DNAs for the parts were synthesized in sense and antisense orientations.

Specifically, as the sense oligo DNAs corresponding to the nucleotide sequence shown by SEQ ID NO: 2, two kinds: a 28-mer: 5'-GCTGGTCAAAATATTGGTGTCTGATTG-3' (common), and a 28-mer: 5'-GATTGCTGAAACACAAAGATATGCTAAT-3', and as the antisense oligo DNAs, four kinds:

a 27-mer: 5'-TGGCTGCAGATTTTCTGTGCTATACTC-3' (common),
a 27-mer: 5'-TGCTTTGCAGCCATATTAGCATATCTT-3',
a 24-mer: 5'-ACAGCCTTTTGAAGCCACCTGGA-3', and
a 24-mer: 5'-ATCGGACTTGTTGTCGGCCTTGAC-3'
were synthesized.

Also, as the sense oligo DNAs corresponding to the nucleotide sequence shown by SEQ ID NO: 4, two kinds: a 28-mer: 5'-GCTGGTCAAAATATTGGTGTCTGATTG-3' (common), and a 28-mer: 5'-GATTGCTCAAACACAGAAGTATGCCTAC-3', and as the antisense oligo DNAs, three kinds:

a 27-mer: 5'-TGGCTGCAGATTTTCTGTGCTATACTC-3' (common),

a 25-mer: 5'-CTTTGCCGCCATGTAGGCATACTTC-3', and

a 24-mer: 5'-TTCCACCTATGGTTGCAGTGTTC-3'

were synthesized.

- 5 Using them as primers, RACE process was carried out with
a commercially available Marathon cDNA Amplification Kit
(manufactured by Clontech) to obtain cDNA fragments having
terminals in 5'-orientation and 3'-orientation,
respectively. Further, a complete length cDNA was obtained
10 by ligating them and cloned into a plasmid vector pCRII
(manufactured by Invitrogen).

Example 9 (Analysis of nucleotide sequence and determination
of amino acid sequence of cDNA clones)

- 15 For two cDNA clones obtained in Example 8, analysis of
nucleotide sequence was carried out with 373A DNA Sequencer
(manufactured by Applied Biosystem) using ABI PRISM Dye
Primer Cycle Sequencing Ready Reaction Kits, Dye Terminator
Cycle Sequencing Kits (manufactured by Applied Biosystems).
20 As a result, it was revealed that the genes of the present
invention were cDNAs having 4,412 bp and 4,359 bp,
respectively (see SEQ ID NOS: 2 and 4).

- Further, based upon said nucleotide sequence, the total
amino acid sequences encoded by the genes of the present
25 invention were determined with GENETYX Gene Analysis

Software (manufactured by SDC, Software Development Co.).

It was revealed that they were proteins having 1,358 and 1,349 amino acid residues, respectively (see SEQ ID NOS: 1 and 3).

5

Example 10 (Construction of aldehyde oxidase expression plasmid for direct introduction)

In order to allow expression of the gene of the present invention derived from maize by introducing in a plant cell, the following direct introduction expression vector for plant, for example, is constructed.

A GUS expression vector pBI221 (manufactured by Clontech) derived from pUC19 is digested by restriction enzymes SmaI and SacI (both being manufactured by Takara Shuzo) and 2.8 Kbp fraction is recovered removing GUS structural gene. The terminus is blunted with T4 DNA polymerase (manufactured by Takara Shuzo). Then, the terminus is treated for de-phosphorylation with bacterial alkaline phosphatase (manufactured by Takara Shuzo).

On the other hand, the complete length cDNA obtained in Example 8 is prepared for an insert gene and the terminus is blunted with T4 DNA polymerase in a similar manner. Afterwards, the both are ligated with T4 DNA ligase (DNA Ligation Kit Ver. 2, manufactured by Takara Shuzo) and used for transforming competent cells of *E. coli* HB101 strain

(manufactured by Takara Shuzo), from which Ampicillin resistant strains are selected. Among the recombinant plasmid amplified from the selected strains, clones in which a coding region for the aldehyde oxidase is inserted in normal orientation in relation to 35S promoter derived from cauliflower mosaic virus and the terminator derived from nopaline synthase and cloned in which said region is inserted in reverse orientation are selected and taken as expression vectors for direct introduction, respectively.

10

Example 11 (Construction of aldehyde oxidase expression plasmid for indirect introduction)

In order to allow expression of the aldehyde oxidase gene derived from maize by introducing in a plant cell, the following indirect introduction expression vector for plant, for example, is constructed.

In a manner similar to that in Example 10, the aldehyde oxidase gene of which the terminus is blunted is prepared for an insert gene. On the other hand, a GUS expression binary vector pBI121 (manufactured by Clontech) derived from pBIN19 is digested by restriction enzymes SmaI and SacI and a fraction is recovered removing GUS structural gene. The terminus is blunted in a similar manner and treated for de-phosphorylation. The both are ligated and used for transforming *E. coli*. The recombinant plasmid are selected

and taken as aldehyde oxidase expression vectors for indirect introduction. Further, the plasmid vectors are transferred to the strain *Agrobacterium tumefaciens* LBA4404 by the tri-parental method (GUS gene fusion system, manufactured
5 by Clontech).

Example 12 (Creation of a transformed plant by introducing aldehyde oxidase expression plasmid; part 1)

The expression vectors for direct introduction
10 obtainable in Example 10 are introduced by a particle gun into an aseptically cultured immature scutellum of rice plant according to a method described in Shimada et al., Ikushugaku Zasshi, 1994, 44 Supplement 1, 66, to obtain transformed rice plants. Similarly, they are introduced by a particle gun
15 into an aseptically cultured immature scutellum of wheat plant according to a method described in Takumi et al., Ikushugaku Zasshi, 1995, 45 Supplement 1, 57, to obtain transformed wheat plants. Similarly, they are introduced by a particle gun into an aseptically cultured immature
20 scutellum of barley plant according to a method described in Hagio et al., Ikushugaku Zasshi, 1994, 44 Supplement 1, 67, to obtain transformed barley plants. Similarly, they are introduced by particle gun into an adventitious embryo of maize according to a method described in M. E. Fromm et al.,
25 Bio/Technology, 1990, 8, 833 - 839, to obtain transformed

maize plants. Further, the expression vectors for direct introduction obtained in Example 10 are introduced by a particle gun into an adventitious embryo of soybean according to a method described in Japanese Patent Application Hei 5 3-291501 to obtain transformed soybean plants.

Example 13 (Creation of a transformed plant by introducing aldehyde oxidase expression plasmid; part 2)

The strains from *Agrobacterium tumefaciens* LBA4404 into 10 which the aldehyde oxidase expression vectors for indirect introduction are introduced, obtainable in Example 11, are infected to an aseptically cultured leaf of tobacco by a method described in Uchimiya, Manual for Plant Gene Manipulation (Method for Producing Transgenic Plants), 15 Published by Kodansha Scientific (ISBN4-06-153513-7), 1990, pages 27 - 33, to obtain transformed tobacco plants. Similarly, they are infected to a petiole of an aseptically cultured seedling of carrot by a method described in N. Pawlicki et al., Plant Cell, Tissue and Organ Culture, 1992, 20 31, 129 - 139, to obtain transformed carrot plants. Further, they are infected to a hypocotyl or cotyledon of an aseptically cultured seedling of *Lotus corniculatus* by a method described in Nagasawa et al., Ikushugaku Zasshi, 1995, 45 Supplement 1, 143, to obtain transformed *Lotus* 25 *corniculatus* plants. Similarly, they are infected to an

SEQ ID NO: 1

SEQUENCE LENGTH: 1,358

SEQUENCE TYPE: Amino acid

5 TOPOLOGY: linear

MOLECULE TYPE: protein

ORIGINAL SOURCE OF SEQUENCE

ORGANISM: maize (*Zea mays* L.)

STRAIN: cultivar: Golden Cross Bantam 70

10 SEQUENCE DESCRIPTION

5 10 15
Met Gly Lys Glu Ala Gly Ala Ala Glu Ser Ser Thr Val Val Leu Ala
20 25 30
Val Asn Gly Lys Arg Tyr Glu Ala Ala Gly Val Ala Pro Ser Thr Ser
15 35 40 45
Leu Leu Glu Phe Leu Arg Thr Gln Thr Pro Val Arg Gly Pro Lys Leu
50 55 60
Gly Cys Gly Glu Gly Gly Cys Gly Ala Cys Val Val Leu Val Ser Lys
65 70 75 80
20 Tyr Asp Pro Ala Thr Asp Glu Val Thr Glu Phe Ser Ala Ser Ser Cys
85 90 95
Leu Thr Leu Leu His Ser Val Asp Arg Cys Ser Val Thr Thr Ser Glu
100 105 110
Gly Ile Gly Asn Thr Arg Asp Gly Tyr His Pro Val Gln Gln Arg Leu
25 115 120 125

Ser Gly Phe His Ala Ser Gln Cys Gly Phe Cys Thr Pro Gly Met Cys
 130 135 140
 Met Ser Ile Phe Ser Ala Leu Val Lys Ala Asp Asn Lys Ser Asp Arg
 145 150 155 160
 5 Pro Asp Pro Pro Ala Gly Phe Ser Lys Ile Thr Thr Ser Glu Ala Glu
 165 170 175
 Lys Ala Val Ser Gly Asn Leu Cys Arg Cys Thr Gly Tyr Arg Pro Ile
 180 185 190
 Val Asp Thr Cys Lys Ser Phe Ala Ser Asp Val Asp Leu Glu Asp Leu
 10 195 200 205
 Gly Leu Asn Cys Phe Trp Lys Lys Gly Glu Glu Pro Ala Glu Val Ser
 210 215 220
 Arg Leu Pro Gly Tyr Asn Ser Gly Ala Val Cys Thr Phe Pro Glu Phe
 225 230 235 240
 15 Leu Lys Ser Glu Ile Lys Ser Thr Met Lys Gln Val Asn Asp Val Pro
 245 250 255
 Ile Ala Ala Ser Gly Asp Gly Trp Tyr His Pro Lys Ser Ile Glu Glu
 260 265 270
 Leu His Arg Leu Phe Asp Ser Ser Trp Phe Asp Asp Ser Ser Val Lys
 20 275 280 285
 Ile Val Ala Ser Asn Thr Gly Ser Gly Val Tyr Lys Asp Gln Asp Leu
 290 295 300
 Tyr Asp Lys Tyr Ile Asp Ile Lys Gly Ile Pro Glu Leu Ser Val Ile
 305 310 315 320
 25 Asn Lys Asn Asp Lys Ala Ile Glu Leu Gly Ser Val Val Ser Ile Ser

325 330 335
 Lys Ala Ile Glu Val Leu Ser Asp Gly Asn Leu Val Phe Arg Lys Ile
 340 345 350
 Ala Asp His Leu Asn Lys Val Ala Ser Pro Phe Val Arg Asn Thr Ala
 5 355 360 365
 Thr Ile Gly Gly Asn Ile Met Met Ala Gln Arg Leu Pro Phe Glu Ser
 370 375 380
 Asp Val Ala Thr Val Leu Leu Ala Ala Gly Ser Thr Val Thr Val Gln
 385 390 395 400
 10 Val Ala Ser Lys Arg Leu Cys Phe Thr Leu Glu Glu Phe Leu Glu Gln
 405 410 415
 Pro Pro Cys Asp Ser Arg Thr Leu Leu Leu Ser Ile Phe Ile Pro Glu
 420 425 430
 Trp Gly Ser Asp Tyr Val Thr Phe Glu Thr Phe Arg Ala Ala Pro Arg
 15 435 440 445
 Pro Phe Gly Asn Ala Val Ser Tyr Val Asn Ser Ala Phe Leu Ala Arg
 450 455 460
 Thr Ser Gly Ser Leu Leu Ile Glu Asp Ile Cys Leu Ala Phe Gly Ala
 465 470 475 480
 20 Tyr Gly Val Asp His Ala Ile Arg Ala Lys Lys Val Glu Asp Phe Leu
 485 490 495
 Lys Gly Lys Ser Leu Ser Ser Phe Val Ile Leu Glu Ala Ile Lys Leu
 500 505 510
 Leu Lys Asp Thr Val Ser Pro Ser Glu Gly Thr Thr His His Glu Tyr
 25 515 520 525

Arg Val Ser Leu Ala Val Ser Phe Leu Phe Ser Phe Leu Ser Ser Leu
 530 535 540
 Ala Asn Ser Ser Ser Ala Pro Ser Asn Ile Asp Thr Pro Asn Gly Ser
 545 550 555 560
 5 Tyr Thr His Glu Thr Gly Ser Asn Val Asp Ser Pro Glu Arg His Ile
 565 570 575
 Lys Val Asp Ser Asn Asp Leu Pro Ile Arg Ser Arg Gln Glu Met Val
 580 585 590
 Phe Ser Asp Glu Tyr Lys Pro Val Gly Lys Pro Ile Lys Lys Val Gly
 10 595 600 605
 Ala Glu Ile Gln Ala Ser Gly Glu Ala Val Tyr Val Asp Asp Ile Pro
 610 615 620
 Ala Pro Lys Asp Cys Leu Tyr Gly Ala Phe Ile Tyr Ser Thr His Pro
 625 630 635 640
 15 His Ala His Val Arg Ser Ile Asn Phe Lys Ser Ser Leu Ala Ser Gln
 645 650 655
 Lys Val Ile Thr Val Ile Thr Ala Lys Asp Ile Pro Ser Gly Gly Glu
 660 665 670
 Asn Ile Gly Ser Ser Phe Leu Met Gln Gly Glu Ala Leu Phe Ala Asp
 20 675 680 685
 Pro Ile Ala Glu Phe Ala Gly Gln Asn Ile Gly Val Val Ile Ala Glu
 690 695 700
 Thr Gln Arg Tyr Ala Asn Met Ala Ala Lys Gln Ala Val Val Glu Tyr
 705 710 715 720
 25 Ser Thr Glu Asn Leu Gln Pro Pro Ile Leu Thr Ile Glu Asp Ala Ile

	725	730	735
	Gln Arg Asn Ser Tyr Ile Gln Ile Pro Pro Phe Leu Ala Pro Lys Pro		
	740	745	750
	Val Gly Asp Tyr Asn Lys Gly Met Ala Glu Ala Asp His Lys Ile Leu		
5	755	760	765
	Ser Ala Glu Val Lys Leu Glu Ser Gln Tyr Tyr Phe Tyr Met Glu Thr		
	770	775	780
	Gln Ala Ala Leu Ala Ile Pro Asp Glu Asp Asn Cys Ile Thr Ile Tyr		
	785	790	795
10	Ser Ser Thr Gln Met Pro Glu Leu Thr Gln Asn Leu Ile Ala Arg Cys		
	805	810	815
	Leu Gly Ile Pro Phe His Asn Val Arg Val Ile Ser Arg Arg Val Gly		
	820	825	830
	Gly Gly Phe Gly Gly Lys Ala Met Lys Ala Thr His Thr Ala Cys Ala		
15	835	840	845
	Cys Ala Leu Ala Ala Phe Lys Leu Arg Arg Pro Val Arg Met Tyr Leu		
	850	855	860
	Asp Arg Lys Thr Asp Met Ile Met Ala Gly Gly Arg His Pro Met Lys		
	865	870	875
20	Ala Lys Tyr Ser Val Gly Phe Lys Ser Asp Gly Lys Ile Thr Ala Leu		
	885	890	895
	His Leu Asp Leu Gly Ile Asn Ala Gly Ile Ser Pro Asp Val Ser Pro		
	900	905	910
	Leu Met Pro Arg Ala Ile Ile Gly Ala Leu Lys Lys Tyr Asn Trp Gly		
25	915	920	925

Thr Leu Glu Phe Asp Thr Lys Val Cys Lys Thr Asn Val Ser Ser Lys
 930 935 940
 Ser Ala Met Arg Ala Pro Gly Asp Val Gln Gly Ser Phe Ile Ala Glu
 945 950 955 960
 5 Ala Ile Ile Glu His Val Ala Ser Ala Leu Ala Leu Asp Thr Asn Thr
 965 970 975
 Val Arg Arg Lys Asn Leu His Asp Phe Glu Ser Leu Glu Val Phe Tyr
 980 985 990
 Gly Glu Ser Ala Gly Glu Ala Ser Thr Tyr Ser Leu Val Ser Met Phe
 10 995 1000 1005
 Asp Lys Leu Ala Leu Ser Pro Glu Tyr Gln His Arg Ala Ala Met Ile
 1010 1015 1020
 Glu Gln Phe Asn Ser Ser Asn Lys Trp Lys Lys Arg Gly Ile Ser Cys
 1025 1030 1035 1040
 15 Val Pro Ala Thr Tyr Glu Val Asn Leu Arg Pro Thr Pro Gly Lys Val
 1045 1050 1055
 Ser Ile Met Asn Asp Gly Ser Ile Ala Val Glu Val Gly Gly Ile Glu
 1060 1065 1070
 Ile Gly Gln Gly Leu Trp Thr Lys Val Lys Gln Met Thr Ala Phe Gly
 20 1075 1080 1085
 Leu Gly Gln Leu Cys Pro Asp Gly Gly Glu Cys Leu Leu Asp Lys Val
 1090 1095 1100
 Arg Val Ile Gln Ala Asp Thr Leu Ser Leu Ile Gln Gly Gly Met Thr
 1105 1110 1115 1120
 25 Ala Gly Ser Thr Thr Ser Glu Thr Ser Cys Glu Thr Val Arg Gln Ser

	1125	1130	1135
	Cys Val Ala Leu Val Glu Lys Leu Asn Pro Ile Lys Glu Ser Leu Glu		
	1140	1145	1150
	Ala Lys Ser Asn Thr Val Glu Trp Ser Ala Leu Ile Ala Gln Ala Ser		
5	1155	1160	1165
	Met Ala Ser Val Asn Leu Ser Ala Gln Pro Tyr Trp Thr Pro Asp Pro		
	1170	1175	1180
	Ser Phe Lys Ser Tyr Leu Asn Tyr Gly Ala Gly Thr Ser Glu Val Glu		
	1185	1190	1195
	1200		
10	Val Asp Ile Leu Thr Gly Ala Thr Thr Ile Leu Arg Ser Asp Leu Val		
	1205	1210	1215
	Tyr Asp Cys Gly Gln Ser Leu Asn Pro Ala Val Asp Leu Gly Gln Ile		
	1220	1225	1230
	Glu Gly Cys Phe Val Gln Gly Ile Gly Phe Phe Thr Asn Glu Asp Tyr		
15	1235	1240	1245
	Lys Thr Asn Ser Asp Gly Leu Val Ile His Asp Gly Thr Trp Thr Tyr		
	1250	1255	1260
	Lys Ile Pro Thr Val Asp Asn Ile Pro Lys Glu Phe Asn Val Glu Met		
	1265	1270	1275
	1280		
20	Phe Asn Ser Ala Pro Asp Lys Lys Arg Val Leu Ser Ser Lys Ala Ser		
	1285	1290	1295
	Gly Glu Pro Pro Leu Val Leu Ala Thr Ser Val His Cys Ala Met Arg		
	1300	1305	1310
	Glu Ala Ile Arg Ala Ala Arg Lys Glu Phe Ser Val Ser Thr Ser Pro		
25	1315	1320	1325

Ala Lys Ser Ala Val Thr Phe Gln Met Asp Val Pro Ala Thr Met Pro

1330

1335

1340

Val Val Lys Glu Leu Cys Gly Leu Asp Val Val Glu Arg Tyr Leu Glu

1345

1350

1355

5 Asn Val Ser Ala Ala Ser Ala Gly Pro Asn Thr Ala Lys Ala

Variable	Mean	SD	Min	Max
Age	35.2	12.5	18	65
Gender	1.2	0.4	1	2
Marital status	1.5	0.5	1	3
Education	12.8	2.1	9	16
Income	1.8	0.6	1	3
Occupation	1.5	0.5	1	3
Health status	1.2	0.4	1	2
Life satisfaction	4.5	1.2	1	7
Stress level	3.2	1.5	1	6
Work-life balance	2.8	1.1	1	5
Family support	1.8	0.7	1	3
Community involvement	1.5	0.6	1	3
Volunteer work	1.2	0.5	1	2
Charitable donations	1.0	0.4	1	2
Political participation	1.5	0.6	1	3
Civic engagement	1.8	0.7	1	3
Environmental awareness	1.2	0.5	1	2
Social responsibility	1.5	0.6	1	3
Ethical consumption	1.8	0.7	1	3
Workplace diversity	1.5	0.6	1	3
Employee engagement	1.2	0.5	1	2
Organizational commitment	1.8	0.7	1	3
Job satisfaction	1.5	0.6	1	3
Turnover intention	1.2	0.5	1	2
Organizational citizenship	1.8	0.7	1	3
Prosocial behavior	1.5	0.6	1	3
Workplace safety	1.2	0.5	1	2
Health and safety	1.8	0.7	1	3
Environmental impact	1.5	0.6	1	3
Social impact	1.2	0.5	1	2
Economic impact	1.8	0.7	1	3
Overall well-being	1.5	0.6	1	3

SEQ ID NO: 2

SEQUENCE LENGTH: 4,412

SEQUENCE TYPE: Nucleic acid

5 STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

ORIGINAL SOURCE OF SEQUENCE

ORGANISM: maize (*Zea mays* L.)

10 STRAIN: cultivar: Golden Cross Bantam 70

FEATURES OF SEQUENCE:

KEY: CDS

LOCATION: 46..4120 (including termination codon)

IDENTIFICATION METHOD: E

15 SEQUENCE DESCRIPTION

GTG CTG TGT TGT GCT GTG CTG CGT GCT GTG GAG GGG GAG GAG GAG ATG 48

GGG AAG GAG GCA GGG GCA GCG GAG TCG TCG ACG GTG GTG CTG GCC GTC 96

AAC GGC AAG CGC TAC GAG GCG GCC GGC GTG GCT CCG TCC ACG TCG CTG 144

CTG GAG TTC CTC CGC ACC CAG ACG CCC GTC AGA GGC CCC AAG CTC GGC 192

20 TGC GGC GAA GGT GGC TGC GGT GCA TGC GTG GTC CTC GTC TCC AAG TAC 240

GAC CCG GCC ACG GAC GAG GTG ACC GAG TTC TCT GCC AGC TCC TGC CTG 288

ACG CTG CTC CAC AGC GTG GAC CGC TGC TCA GTG ACC ACC AGC GAG GGA 336

ATC GGC AAC ACC AGG GAT GGC TAC CAC CCC GTG CAG CAG CGC CTC TCC 384

GGC TTC CAC GCC TCG CAG TGC GGC TTC TGC ACA CCC GGC ATG TGC ATG 432

25 TCC ATC TTC TCC GCC CTT GTC AAG GCC GAC AAC AAG TCC GAT CGC CCG 480

GAC CCT CCT GCT GGC TTC TCC AAG ATC ACT ACC TCG GAG GCA GAG AAG 528
 GCT GTC TCG GGC AAC CTT TGT CGT TGC ACC GGA TAC AGA CCC ATT GTT 576
 GAC ACC TGC AAA AGC TTT GCC TCT GAT GTT GAC CTC GAG GAC CTA GGC 624
 CTC AAC TGT TTC TGG AAG AAG GGC GAA GAA CCT GCA GAA GTC AGC AGG 672
 5 CTG CCG GGG TAC AAC AGC GGT GCC GTC TGC ACC TTT CCA GAG TTT CTC 720
 AAA TCC GAA ATC AAG TCT ACT ATG AAG CAG GTG AAC GAT GTC CCC ATT 768
 GCA GCC TCA GGT GAT GGC TGG TAC CAT CCT AAG AGC ATT GAA GAG CTT 816
 CAC AGG TTG TTT GAT TCC AGC TGG TTT GAT GAC AGT TCT GTG AAG ATT 864
 GTT GCT TCA AAC ACT GGG TCT GGA GTG TAC AAG GAT CAG GAC CTC TAC 912
 10 GAC AAG TAC ATT GAC ATC AAA GGA ATC CCA GAG CTT TCA GTC ATC AAT 960
 AAA AAC GAC AAA GCA ATT GAG CTT GGA TCA GTT GTG TCC ATC TCT AAA 1008
 GCT ATT GAA GTG CTG TCA GAT GGA AAT TTG GTC TTC AGA AAG ATT GCT 1056
 GAT CAC CTC AAC AAA GTG GCT TCA CCG TTT GTT CGG AAC ACT GCA ACC 1104
 ATA GGA GGA AAC ATA ATG ATG GCA CAA AGG TTG CCA TTT GAA TCG GAT 1152
 15 GTT GCA ACC GTG CTC CTA GCT GCG GGT TCG ACA GTC ACA GTC CAG GTG 1200
 GCT TCC AAA AGG CTG TGC TTC ACT CTG GAG GAA TTC TTG GAA CAA CCT 1248
 CCA TGT GAT TCT AGG ACC CTG CTG CTG AGC ATA TTT ATC CCA GAA TGG 1296
 GGT TCA GAC TAT GTC ACC TTT GAG ACT TTC CGA GCC GCC CCA CGA CCA 1344
 TTT GGA AAT GCT GTC TCT TAT GTA AAC TCT GCT TTC TTG GCA AGG ACA 1392
 20 TCA GGC AGC CTT CTA ATT GAG GAT ATA TGC TTG GCA TTT GGT GCC TAC 1440
 GGA GTC GAT CAT GCC ATC AGA GCT AAG AAG GTT GAA GAT TTC TTG AAG 1488
 GGA AAA TCG CTG AGC TCA TTT GTG ATA CTT GAA GCA ATT AAA CTA CTC 1536
 AAA GAT ACC GTT TCA CCA TCA GAA GGC ACT ACA CAT CAT GAA TAC AGG 1584
 GTC AGC TTG GCT GTC AGT TTC TTG TTC AGT TTC TTA TCT TCC CTT GCC 1632
 25 AAC AGT TCG AGT GCA CCA TCA AAT ATT GAT ACT CCC AAT GGG TCA TAT 1680

ACT CAT GAA ACT GGT AGC AAT GTG GAC TCA CCT GAG AGG CAT ATT AAG 1728

GTT GAC AGC AAT GAT TTG CCA ATT CGT TCA AGA CAA GAA ATG GTT TTC 1776

AGC GAT GAG TAC AAG CCT GTT GGC AAG CCG ATC AAG AAA GTC GGG GCA 1824

GAG ATC CAA GCA TCA GGG GAG GCT GTG TAC GTT GAT GAT ATC CCT GCT 1872

5 CCC AAG GAT TGC CTC TAT GGA GCA TTT ATC TAC AGC ACA CAT CCT CAT 1920

GCT CAT GTG AGA AGT ATC AAC TTC AAA TCA TCC TTG GCT TCA CAG AAG 1968

GTC ATC ACA GTT ATA ACC GCA AAG GAT ATT CCA AGC GGT GGA GAA AAT 2016

ATT GGA AGC AGC TTC CTG ATG CAA GGA GAA GCA CTA TTT GCA GAT CCA 2064

ATC GCT GAA TTT GCT GGT CAA AAT ATT GGT GTC GTG ATT GCT GAA ACA 2112

10 CAA AGA TAT GCT AAT ATG GCT GCA AAG CAA GCT GTT GTT GAG TAT AGC 2160

ACA GAA AAT CTG CAG CCA CCA ATT CTG ACA ATA GAA GAT GCC ATC CAA 2208

AGA AAC AGC TAC ATC CAA ATT CCC CCA TTT TTA GCT CCA AAG CCA GTT 2256

GGT GAC TAC AAC AAA GGG ATG GCT GAA GCA GAC CAC AAG ATT CTA TCA 2304

GCA GAG GTA AAA CTT GAA TCC CAG TAC TAC TTC TAC ATG GAA ACT CAA 2352

15 GCA GCA CTA GCG ATT CCT GAT GAA GAT AAC TGC ATA ACA ATC TAT TCC 2400

TCG ACA CAA ATG CCT GAG CTC ACA CAA AAT TTG ATA GCA AGG TGT CTT 2448

GGC ATT CCA TTT CAC AAT GTC CGT GTC ATC AGC AGA AGA GTA GGA GGA 2496

GGC TTT GGT GGA AAG GCA ATG AAA GCA ACG CAT ACT GCA TGT GCA TGT 2544

GCC CTT GCT GCC TTC AAG CTG CGG CGT CCA GTT AGG ATG TAC CTC GAT 2592

20 CGC AAG ACG GAC ATG ATA ATG GCT GGA GGG AGA CAT CCA ATG AAG GCG 2640

AAG TAC TCT GTT GGG TTC AAG TCA GAT GGC AAG ATC ACA GCC TTG CAC 2688

CTA GAT CTT GGA ATC AAT GCT GGA ATA TCA CCA GAT GTG AGT CCA TTG 2736

ATG CCA CGT GCT ATC ATA GGA GCT CTC AAA AAG TAC AAC TGG GGC ACT 2784

CTT GAA TTT GAC ACC AAG GTC TGC AAG ACA AAT GTC TCA TCA AAG TCA 2832

25 GCA ATG CGA GCT CCT GGA GAT GTG CAG GGC TCT TTC ATC GCT GAA GCC 2880

ATC ATC GAG CAT GTT GCC TCA GCA CTC GCA CTA GAC ACT AAC ACC GTC 2928
 AGG AGG AAG AAC CTT CAT GAT TTT GAA AGC CTT GAA GTT TTC TAT GGA 2976
 GAA AGT GCA GGT GAA GCT TCT ACA TAC AGC CTG GTT TCC ATG TTT GAC 3024
 AAG CTG GCC TTG TCT CCA GAA TAC CAG CAC AGG GCT GCA ATG ATT GAG 3072
 5 CAG TTC AAT AGC AGC AAC AAA TGG AAG AAA CGC GGC ATT TCT TGT GTG 3120
 CCA GCC ACT TAT GAG GTT AAT CTT CGA CCA ACT CCA GGC AAG GTG TCA 3168
 ATC ATG AAT GAT GGT TCC ATC GCT GTC GAG GTT GGA GGA ATT GAG ATA 3216
 GGT CAA GGA TTG TGG ACT AAA GTG AAG CAG ATG ACG GCC TTT GGA CTG 3264
 GGA CAG CTG TGT CCT GAT GGT GGC GAA TGC CTT CTG GAC AAG GTT CGG 3312
 10 GTT ATC CAG GCA GAC ACA TTA AGC CTG ATC CAA GGA GGT ATG ACT GCT 3360
 GGG AGC ACC ACT TCT GAA ACT AGC TGT GAA ACA GTT CGG CAA TCT TGT 3408
 GTT GCA CTG GTT GAG AAG CTG AAC CCT ATC AAG GAG AGT CTC GAA GCT 3456
 AAG TCC AAC ACA GTG GAA TGG AGT GCC TTG ATT GCT CAG GCA AGC ATG 3504
 GCG AGT GTG AAC CTA TCA GCA CAG CCG TAC TGG ACT CCT GAT CCA TCT 3552
 15 TTC AAG AGC TAC TTG AAC TAC GGA GCT GGC ACC AGT GAG GTG GAA GTT 3600
 GAT ATC CTA ACA GGA GCA ACC ACA ATT CTG CGA AGC GAC CTG GTG TAT 3648
 GAC TGC GGG CAG AGC CTA AAC CCT GCT GTA GAC TTG GGC CAG ATC GAG 3696
 GGC TGC TTT GTC CAA GGA ATA GGG TTC TTC ACG AAC GAG GAC TAC AAG 3744
 ACG AAT TCC GAC GGG TTG GTC ATC CAC GAC GGC ACA TGG ACG TAC AAG 3792
 20 ATC CCC ACG GTG GAT AAT ATC CCG AAG GAG TTC AAT GTT GAG ATG TTT 3840
 AAC AGC GCC CCT GAC AAG AAG CGT GTC CTA TCT TCC AAA GCG TCG GGC 3888
 GAG CCG CCG CTG GTT CTC GCA ACC TCG GTG CAC TGC GCG ATG AGG GAG 3936
 GCC ATC AGG GCG GCG AGG AAG GAG TTC TCG GTC AGC ACC AGC CCC GCG 3984
 AAA TCC GCC GTC ACA TTC CAG ATG GAC GTG CCG GCG ACG ATG CCT GTC 4032
 25 GTC AAG GAG CTC TGC GGC CTC GAC GTC GTG GAG AGG TAC CTC GAG AAC 4080

	GTG TCT GCC GCC AGT GCC GGC CCA AAC ACA GCG AAA GCA TAG ATC CAG	4128
	CAG GCC TCA GGG TGC AGT CGG CGC ACT GCC AGA GAT GAT GTG TGC TGC	4176
	CCT GAT GTA CAG ACA GTA CAG TAC AGA GGA GAG AGA ATT GGG GGA ACT	4224
	CAG GAA CTG CGA GGA GCG ATG AAC AGT ATA TAG AGT GAA AAA TAA AAG	4272
5	TGC TTC GTA CTA ATA ATC ACT AGA AAA AAT TAT GCA CAT CTC CCA CGC	4320
	ACT ACC GGC ACG ACT GTT GAA TAT TTT GTA AAA TAA GAT GTC ATA AGC	4368
	TAT TTA TTT TCT GTA AAA AAA AAA AAA AAA AAA AAA AAA AA	4412

SEQ ID NO: 3

SEQUENCE LENGTH: 1,349

SEQUENCE TYPE: Amino acid

5 TOPOLOGY: linear

MOLECULE TYPE: protein

ORIGINAL SOURCE OF SEQUENCE

ORGANISM: maize (*Zea mays* L.)

STRAIN: cultivar: Golden Cross Bantam 70

10 SEQUENCE DESCRIPTION

5 10 15
Met Glu Met Gly Lys Ala Ala Ala Val Val Leu Ala Val Asn Gly Lys
20 25 30
Arg Tyr Glu Ala Ala Gly Val Asp Pro Ser Thr Thr Leu Leu Glu Phe
15 35 40 45
Leu Arg Thr His Thr Pro Val Arg Gly Pro Lys Leu Gly Cys Gly Glu
50 55 60
Gly Gly Cys Gly Ala Cys Val Val Leu Val Ser Lys Tyr Asp Pro Ala
65 70 75 80
20 Thr Asp Glu Val Thr Glu Phe Ser Ala Ser Ser Cys Leu Thr Leu Leu
85 90 95
His Ser Val Asp Arg Cys Ser Val Thr Thr Ser Glu Gly Ile Gly Asn
100 105 110
Thr Lys Asp Gly Tyr His Pro Val Gln Gln Arg Leu Ser Gly Phe His
25 115 120 125

Ala Ser Gln Cys Gly Phe Cys Thr Pro Gly Met Cys Met Ser Ile Phe
 130 135 140
 Ser Ala Leu Val Lys Ala Asp Lys Ala Ala Asn Arg Pro Ala Pro Pro
 145 150 155 160
 5 Ala Gly Phe Ser Lys Leu Thr Ser Ser Glu Ala Glu Lys Ala Val Ser
 165 170 175
 Gly Asn Leu Cys Arg Cys Thr Gly Tyr Arg Pro Ile Val Asp Ala Cys
 180 185 190
 Lys Ser Phe Ala Ala Asp Val Asp Leu Glu Asp Leu Gly Leu Asn Cys
 10 195 200 205
 Phe Trp Lys Lys Gly Asp Glu Pro Ala Asp Val Ser Lys Leu Pro Gly
 210 215 220
 Tyr Asn Ser Gly Asp Val Cys Thr Phe Pro Asp Phe Leu Lys Ser Glu
 225 230 235 240
 15 Met Lys Ser Ser Ile Gln Gln Ala Asn Ser Ala Pro Val Pro Val Ser
 245 250 255
 Asp Asp Gly Trp Tyr Arg Pro Arg Ser Ile Asp Glu Leu His Arg Leu
 260 265 270
 Phe Gln Ser Ser Ser Phe Asp Glu Asn Ser Val Lys Ile Val Ala Ser
 20 275 280 285
 Asn Thr Gly Ser Gly Val Tyr Lys Asp Gln Asp Leu Tyr Asp Lys Tyr
 290 295 300
 Ile Asp Ile Lys Gly Ile Pro Glu Leu Ser Val Ile Asn Arg Asn Asp
 305 310 315 320
 25 Lys Gly Ile Glu Leu Gly Ser Val Val Ser Ile Ser Lys Ala Ile Glu

325 330 335
 Val Leu Ser Asp Gly Asn Leu Val Phe Arg Lys Ile Ala Gly His Leu
 340 345 350
 Asn Lys Val Ala Ser Pro Phe Val Arg Asn Thr Ala Thr Ile Gly Gly
 5 355 360 365
 Asn Ile Val Met Ala Gln Arg Leu Pro Phe Ala Ser Asp Ile Ala Thr
 370 375 380
 Ile Leu Leu Ala Ala Gly Ser Thr Val Thr Ile Gln Val Ala Ser Lys
 385 390 395 400
 10 Arg Leu Cys Phe Thr Leu Glu Glu Phe Leu Gln Gln Pro Pro Cys Asp
 405 410 415
 Ser Arg Thr Leu Leu Leu Ser Ile Phe Ile Pro Glu Trp Gly Ser Asn
 420 425 430
 Asp Val Thr Phe Glu Thr Phe Arg Ala Ala Pro Arg Pro Leu Gly Asn
 15 435 440 445
 Ala Val Ser Tyr Val Asn Ser Ala Phe Leu Ala Arg Thr Ser Leu Asp
 450 455 460
 Ala Ala Ser Lys Asp His Leu Ile Glu Asp Ile Cys Leu Ala Phe Gly
 465 470 475 480
 20 Ala Tyr Gly Ala Asp His Ala Ile Arg Ala Arg Lys Val Glu Asp Tyr
 485 490 495
 Leu Lys Gly Lys Thr Val Ser Ser Ser Val Ile Leu Glu Ala Val Arg
 500 505 510
 Leu Leu Lys Gly Ser Ile Lys Pro Ser Glu Gly Ser Thr His Pro Glu
 25 515 520 525

Tyr Arg Ile Ser Leu Ala Val Ser Phe Leu Phe Thr Phe Leu Ser Ser
 530 535 540
 Leu Ala Asn Ser Leu Asn Glu Ser Ala Lys Val Ser Gly Thr Asn Glu
 545 550 555 560
 5 His Ser Pro Glu Lys Gln Leu Lys Leu Asp Ile Asn Asp Leu Pro Ile
 565 570 575
 Arg Ser Arg Gln Glu Ile Phe Phe Thr Asp Ala Tyr Lys Pro Val Gly
 580 585 590
 Lys Ala Ile Lys Lys Ala Gly Val Glu Ile Gln Ala Ser Gly Glu Ala
 10 595 600 605
 Val Tyr Val Asp Asp Ile Pro Ala Pro Lys Asp Cys Leu Tyr Gly Ala
 610 615 620
 Phe Ile Tyr Ser Thr His Pro His Ala His Val Lys Ser Ile Asn Phe
 625 630 635 640
 15 Lys Pro Ser Leu Ala Ser Gln Lys Ile Ile Thr Val Ile Thr Ala Lys
 645 650 655
 Asp Ile Pro Ser Gly Gly Gln Asn Val Gly Tyr Ser Phe Pro Met Ile
 660 665 670
 Gly Glu Glu Ala Leu Phe Ala Asp Pro Val Ala Glu Phe Ala Gly Gln
 20 675 680 685
 Asn Ile Gly Val Val Ile Ala Gln Thr Gln Lys Tyr Ala Tyr Met Ala
 690 695 700
 Ala Lys Gln Ala Ile Ile Glu Tyr Ser Thr Glu Asn Leu Gln Pro Pro
 705 710 715 720
 25 Ile Leu Thr Ile Glu Asp Ala Ile Glu Arg Ser Ser Phe Phe Gln Thr

725 730 735
 Leu Pro Phe Val Ala Pro Lys Pro Val Gly Asp Tyr Asp Lys Gly Met
 740 745 750
 Ser Glu Ala Asp His Lys Ile Leu Ser Ala Glu Val Lys Ile Glu Ser
 5 755 760 765
 Gln Tyr Phe Phe Tyr Met Glu Pro Gln Val Ala Leu Ala Ile Pro Asp
 770 775 780
 Glu Asp Asn Cys Ile Thr Ile Tyr Phe Ser Thr Gln Leu Pro Glu Ser
 785 790 795 800
 10 Thr Gln Asn Val Val Ala Lys Cys Val Gly Ile Pro Phe His Asn Val
 805 810 815
 Arg Val Ile Thr Arg Arg Val Gly Gly Gly Phe Gly Gly Lys Ala Leu
 820 825 830
 Lys Ser Met His Val Ala Cys Ala Cys Ala Val Ala Ala Leu Lys Leu
 15 835 840 845
 Gln Arg Pro Val Arg Met Tyr Leu Asp Arg Lys Thr Asp Met Ile Met
 850 855 860
 Ala Gly Gly Arg His Pro Met Lys Val Lys Tyr Ser Val Gly Phe Lys
 865 870 875 880
 20 Ser Asn Gly Lys Ile Thr Ala Leu His Leu Asp Leu Gly Ile Asn Gly
 885 890 895
 Gly Ile Ser Pro Asp Met Ser Pro Met Ile Ala Ala Pro Val Ile Gly
 900 905 910
 Ser Leu Lys Lys Tyr Asn Trp Gly Asn Leu Ala Phe Asp Thr Lys Val
 25 915 920 925

Cys Lys Thr Asn Val Ser Ser Lys Ser Ser Met Arg Ala Pro Gly Asp
 930 935 940
 Ala Gln Gly Ser Phe Ile Ala Glu Ala Ile Ile Glu His Val Ala Ser
 945 950 955 960
 5 Ala Leu Ser Ala Asp Thr Asn Thr Ile Arg Arg Lys Asn Leu His Asp
 965 970 975
 Phe Glu Ser Leu Ala Val Phe Phe Gly Asp Ser Ala Gly Glu Ala Ser
 980 985 990
 Thr Tyr Ser Leu Val Thr Met Phe Asp Lys Leu Ala Ser Ser Pro Glu
 10 995 1000 1005
 Tyr Gln His Arg Ala Glu Met Val Glu Gln Phe Asn Arg Ser Asn Lys
 1010 1015 1020
 Trp Lys Lys Arg Gly Ile Ser Cys Val Pro Val Thr Tyr Glu Val Gln
 1025 1030 1035 1040
 15 Leu Arg Pro Thr Pro Gly Lys Val Ser Ile Met Asn Asp Gly Ser Ile
 1045 1050 1055
 Ala Val Glu Val Gly Gly Val Glu Leu Gly Gln Gly Leu Trp Thr Lys
 1060 1065 1070
 Val Lys Gln Met Thr Ala Phe Gly Leu Gly Gln Leu Cys Pro Gly Gly
 20 1075 1080 1085
 Gly Glu Ser Leu Leu Asp Lys Val Arg Val Ile Gln Ala Asp Thr Leu
 1090 1095 1100
 Ser Met Ile Gln Gly Gly Val Thr Gly Gly Ser Thr Thr Ser Glu Thr
 1105 1110 1115 1120
 25 Ser Cys Glu Ala Val Arg Lys Ser Cys Val Ala Leu Val Glu Ser Leu

1125 1130 1135
 Lys Pro Ile Lys Glu Asn Leu Glu Ala Lys Thr Gly Thr Val Glu Trp
 1140 1145 1150
 Ser Ala Leu Ile Ala Gln Ala Ser Met Ala Ser Val Asn Leu Ser Ala
 5 1155 1160 1165
 His Ala Tyr Trp Thr Pro Asp Pro Thr Phe Thr Ser Tyr Leu Asn Tyr
 1170 1175 1180
 Gly Ala Gly Thr Ser Glu Val Glu Ile Asp Val Leu Thr Gly Ala Thr
 1185 1190 1195 1200
 10 Thr Ile Leu Arg Ser Asp Leu Val Tyr Asp Cys Gly Gln Ser Leu Asn
 1205 1210 1215
 Pro Ala Val Asp Leu Gly Gln Val Glu Gly Ala Phe Val Gln Gly Val
 1220 1225 1230
 Gly Phe Phe Thr Asn Glu Glu Tyr Ala Thr Asn Ser Asp Gly Leu Val
 15 1235 1240 1245
 Ile His Asp Gly Thr Trp Thr Tyr Lys Ile Pro Thr Val Asp Thr Ile
 1250 1255 1260
 Pro Lys Gln Phe Asn Val Glu Leu Ile Asn Ser Ala Arg Asp Gln Lys
 1265 1270 1275 1280
 20 Arg Val Leu Ser Ser Lys Ala Ser Gly Glu Pro Pro Leu Leu Leu Ala
 1285 1290 1295
 Ser Ser Val His Cys Ala Met Arg Glu Ala Ile Arg Ala Ala Arg Lys
 1300 1305 1310
 Glu Phe Ser Val Cys Thr Gly Pro Ala Asn Ser Ala Ile Thr Phe Gln
 25 1315 1320 1325

Met Asp Val Pro Ala Thr Met Pro Val Val Lys Glu Leu Cys Gly Leu

1330

1335

1340

Asp Val Val Glu Arg Tyr Leu Glu Ser Val Ser Ala Ala Ser Pro Thr

1345

5 Asn Thr Ala Lys Ala

[illegible]

SEQ ID NO: 4

SEQUENCE LENGTH: 4,359

SEQUENCE TYPE: Nucleic acid

5 STRANDEDNESS: single

TOPOLOGY: linear

MOLECULE TYPE: cDNA to mRNA

ORIGINAL SOURCE OF SEQUENCE

ORGANISM: maize (*Zea mays* L.)

10 STRAIN: cultivar: Golden Cross Bantam 70

FEATURES OF SEQUENCE:

KEY: CDS

LOCATION: 91..4138 (including termination codon)

IDENTIFICATION METHOD: E

15 SEQUENCE DESCRIPTION

CCG GCT CTC TCG GTG CAG ACG TCC GGG ACT AGT ACG TGG ATC GGG CCG 48

GGG GCA ACT CGA GTC GTC AAG AAG GCT GCT ACC TGC TAG AGG ATG GAG 96

ATG GGG AAG GCG GCG GCG GTG GTG CTG GCG GTG AAC GGC AAG CGG TAC 144

GAG GCC GCC GGC GTG GAC CCG TCG ACG ACG CTG CTG GAG TTC CTG CGC 192

20 ACC CAC ACG CCC GTC AGG GGG CCC AAG CTC GGC TGC GGC GAA GGT GGC 240

TGC GGT GCA TGC GTT GTG CTT GTC TCG AAG TAC GAC CCA GCC ACC GAC 288

GAG GTG ACC GAG TTC TCA GCG AGC TCC TGC CTG ACG CTG CTC CAT AGC 336

GTG GAC CGC TGC TCG GTG ACC ACC AGC GAG GGC ATT GGC AAC ACC AAG 384

GAT GGC TAC CAC CCT GTG CAG CAG CGC CTC TCC GGC TTC CAC GCC TCC 432

25 CAG TGC GGT TTC TGC ACG CCC GGC ATG TGC ATG TCC ATC TTC TCT GCG 480

CTT GTC AAA GCC GAC AAG GCG GCC AAC CGG CCA GCC CCA CCG GCC GGC 528
 TTC TCC AAG CTC ACT TCC TCG GAG GCT GAG AAG GCT GTC TCT GGC AAC 576
 CTG TGC CGC TGC ACA GGG TAC AGG CCC ATC GTC GAC GCC TGT AAG AGC 624
 TTC GCA GCC GAT GTT GAT CTT GAG GAC CTG GGC CTC AAC TGC TTC TGG 672
 5 AAG AAG GGT GAT GAG CCT GCA GAT GTC AGC AAG CTG CCA GGC TAC AAC 720
 AGT GGT GAC GTC TGC ACT TTC CCT GAC TTT CTC AAA TCT GAG ATG AAG 768
 TCC TCA ATT CAG CAG GCT AAC AGC GCT CCA GTT CCT GTT TCT GAC GAC 816
 GGC TGG TAC CGT CCT AGG AGC ATT GAC GAG CTT CAC AGG TTG TTT CAA 864
 TCT AGC TCC TTC GAT GAA AAT TCC GTG AAG ATA GTG GCT TCA AAC ACT 912
 10 GGG TCT GGA GTG TAC AAG GAT CAG GAC CTT TAT GAC AAG TAC ATT GAC 960
 ATC AAA GGA ATC CCA GAG CTT TCA GTC ATC AAC AGA AAC GAC AAA GGA 1008
 ATT GAG CTT GGA TCA GTT GTG TCC ATC TCT AAA GCT ATT GAG GTG CTG 1056
 TCA GAT GGA AAT CTC GTC TTC AGA AAG ATT GCT GGT CAC CTG AAC AAA 1104
 GTG GCT TCA CCG TTT GTT CGG AAC ACT GCA ACC ATA GGT GGA AAC ATA 1152
 15 GTC ATG GCA CAA AGA TTG CCA TTC GCA TCG GAC ATT GCA ACC ATA CTA 1200
 CTA GCT GCA GGT TCA ACA GTC ACA ATC CAG GTG GCT TCC AAA AGG CTG 1248
 TGC TTC ACT CTG GAG GAG TTC TTG CAG CAG CCT CCA TGC GAT TCT AGG 1296
 ACC CTG CTG CTG AGC ATA TTT ATC CCG GAA TGG GGC TCA AAT GAT GTC 1344
 ACC TTT GAG ACT TTC CGA GCA GCA CCT CGT CCA CTT GGC AAT GCT GTC 1392
 20 TCA TAT GTC AAT TCA GCT TTC TTG GCA AGG ACT TCA TTG GAT GCA GCA 1440
 TCA AAG GAC CAT CTC ATC GAG GAT ATA TGT CTG GCG TTC GGT GCT TAT 1488
 GGA GCT GAT CAT GCT ATT AGA GCT AGA AAG GTT GAG GAT TAC CTG AAG 1536
 GGC AAA ACA GTG AGC TCG TCT GTC ATA CTT GAA GCT GTT CGG TTG CTT 1584
 AAA GGG TCT ATT AAA CCA TCA GAA GGC TCA ACA CAT CCT GAG TAT AGA 1632
 25 ATT AGC TTG GCT GTC AGT TTC TTG TTT ACC TTC CTA TCC TCC CTT GCC 1680

AAC AGC TTG AAT GAA TCT GCA AAG GTT AGT GGT ACC AAC GAG CAC TCA 1728
CCA GAG AAG CAA CTC AAG TTG GAC ATC AAT GAT TTG CCA ATA CGA TCA 1776
AGA CAA GAA ATA TTT TTC ACT GAT GCA TAT AAG CCA GTT GGC AAA GCA 1824
ATT AAG AAA GCT GGG GTA GAG ATC CAA GCT TCA GGG GAA GCT GTG TAC 1872
5 GTT GAT GAT ATC CCT GCT CCC AAA GAT TGC CTC TAT GGG GCA TTT ATT 1920
TAT AGC ACA CAC CCT CAT GCA CAT GTA AAG TCA ATC AAC TTT AAA CCA 1968
TCT TTG GCT TCA CAG AAG ATC ATC ACA GTT ATC ACT GCA AAG GAT ATT 2016
CCC AGC GGT GGA CAA AAT GTT GGT TAT AGC TTC CCG ATG ATT GGA GAA 2064
GAA GCA CTT TTT GCA GAT CCA GTT GCT GAA TTT GCT GGT CAA AAT ATT 2112
10 GGT GTC GTG ATT GCT CAA ACA CAG AAG TAT GCC TAC ATG GCG GCA AAG 2160
CAA GCC ATC ATT GAG TAT AGC ACA GAA AAT CTG CAG CCA CCA ATT CTG 2208
ACA ATA GAA GAT GCA ATT GAA CGA AGC AGC TTC TTC CAA ACC CTC CCA 2256
TTT GTA GCT CCT AAG CCA GTT GGT GAT TAC GAC AAA GGG ATG TCT GAA 2304
GCT GAT CAC AAG ATT TTA TCG GCA GAG GTA AAA ATT GAA TCC CAA TAC 2352
15 TTT TTC TAC ATG GAG CCA CAA GTG GCG CTA GCT ATT CCT GAT GAA GAT 2400
AAC TGC ATA ACC ATC TAT TTT TCG ACA CAA TTA CCT GAG TCC ACA CAA 2448
AAT GTG GTT GCA AAG TGC GTT GGC ATT CCA TTT CAC AAT GTC CGT GTA 2496
ATC ACC AGA AGG GTC GGA GGA GGC TTT GGT GGA AAA GCA TTG AAA TCA 2544
ATG CAT GTT GCA TGT GCA TGT GCA GTT GCT GCA TTG AAG CTA CAA CGT 2592
20 CCA GTT CGG ATG TAC CTC GAT CGC AAG ACA GAC ATG ATA ATG GCA GGC 2640
GGG CGG CAT CCT ATG AAG GTG AAG TAC TCT GTT GGG TTC AAG TCA AAC 2688
GGC AAG ATC ACA GCC TTA CAT CTT GAT CTT GGG ATC AAT GGT GGA ATA 2736
TCT CCA GAT ATG AGT CCA ATG ATT GCA GCA CCT GTC ATA GGT TCT CTC 2784
AAA AAG TAC AAC TGG GGC AAT CTT GCA TTT GAC ACC AAG GTC TGC AAA 2832
25 ACA AAT GTC TCA TCA AAA TCG TCA ATG AGA GCT CCT GGA GAT GCG CAG 2880

GGC TCT TTC ATT GCT GAA GCC ATC ATC GAG CAT GTT GCC TCG GCA CTT 2928

TCA GCC GAC ACT AAT ACC ATA AGG AGA AAG AAC CTT CAT GAC TTT GAG 2976

AGC CTT GCA GTG TTC TTT GGA GAT AGT GCA GGT GAA GCT TCT ACA TAC 3024

AGC CTT GTC ACC ATG TTC GAT AAA TTG GCC TCC TCT CCA GAA TAC CAG 3072

5 CAC CGA GCT GAA ATG GTG GAA CAA TTC AAC CGA AGC AAC AAG TGG AAG 3120

AAG CGT GGC ATT TCT TGT GTG CCT GTA ACA TAT GAG GTG CAG CTT CGG 3168

CCA ACT CCA GGA AAG GTG TCT ATC ATG AAT GAT GGT TCC ATT GCT GTT 3216

GAG GTT GGA GGG GTT GAG CTA GGC CAA GGG TTG TGG ACA AAA GTG AAG 3264

CAG ATG ACG GCA TTC GGA CTA GGA CAG CTG TGT CCT GGC GGC GGT GAA 3312

10 AGC CTT CTA GAC AAG GTG CGG GTC ATC CAG GCC GAC ACA TTG AGC ATG 3360

ATC CAA GGA GGG GTC ACT GGT GGG AGC ACC ACT TCT GAA ACT AGC TGT 3408

GAA GCA GTT CGT AAG TCG TGT GTT GCA CTC GTC GAG AGC TTG AAG CCA 3456

ATC AAG GAG AAT CTG GAG GCT AAA ACT GGC ACA GTG GAA TGG AGT GCC 3504

TTG ATT GCA CAG GCA AGT ATG GCG AGC GTT AAC TTA TCG GCA CAT GCA 3552

15 TAC TGG ACC CCT GAT CCA ACT TTC ACA AGC TAT TTG AAC TAC GGA GCC 3600

GGC ACT AGC GAG GTG GAA ATT GAT GTC CTG ACA GGA GCA ACA ACA ATT 3648

CTA AGG AGT GAC CTT GTC TAC GAT TGC GGG CAA AGC TTG AAC CCT GCT 3696

GTC GAT TTG GGG CAG GTG GAA GGT GCA TTC GTA CAA GGA GTA GGC TTC 3744

TTC ACA AAC GAG GAG TAC GCA ACC AAC TCT GAC GGG TTG GTC ATC CAC 3792

20 GAT GGC ACA TGG ACG TAC AAG ATC CCC ACG GTC GAC ACC ATC CCA AAG 3840

CAG TTC AAC GTT GAG CTG ATC AAC AGC GCC CGT GAC CAG AAG CGC GTC 3888

CTC TCT TCC AAA GCA TCG GGC GAG CCT CCG CTT CTC CTA GCT TCC TCT 3936

GTG CAC TGC GCA ATG AGG GAG GCC ATC AGG GCC GCC AGG AAA GAA TTC 3984

TCG GTC TGC ACT GGT CCA GCG AAC TCC GCC ATC ACG TTC CAG ATG GAC 4032

25 GTG CCG GCA ACG ATG CCT GTC GTC AAG GAG CTC TGC GGC CTG GAT GTC 4080

	GTT GAG AGG TAC CTG GAG AGC GTG TCG GCT GCC AGC CCA ACA AAC ACC	4128
	GCT AAA GCA TAG ATC CAG TAG GCG CTC TAT CCA TGG TGT GAT GGC TTA	4176
	ATC AAT CTG TAA AAC ACT AAG CGG CGT GAC ATG CCG AGC TTT CAG TGT	4224
	TAG CTA TGA TGT ACA GAA GAA GAG GTA CCA ATG GCG AGT TGT GGC CAT	4272
5	GCG AAT CAG GAG TCA TGA ACC ATT GAG GGG GCA AAT AAA GTA AAT AAG	4320
	TGT TGC GCC GGC GAA AAA AAA AAA AAA AAA AAA AAA AAA	4359

0044144 100397

CLAIMS

1. An aldehyde oxidase gene which is a 4.4 Kbp gene obtainable from a plant and which encodes an amino acid
5 sequence of an enzyme capable of oxidizing an aldehyde compound to a carboxylic acid.

2. The aldehyde oxidase gene according to claim 1, wherein the aldehyde compound is indoleacetaldehyde and the carboxylic acid is indoleacetic acid.

10 3. The aldehyde oxidase gene according to claim 1 or 2 which is derived from maize plant (*Zea mays L.*).

4. The aldehyde oxidase gene according to claim 1 which is a nucleotide sequence encoding an amino acid sequence shown by SEQ ID NO: 1.

15 5. The aldehyde oxidase gene according to claim 4 which has a nucleotide sequence shown by SEQ ID NO: 2 (loci of CDS being 46..4120).

6. The aldehyde oxidase gene according to claim 1 which is a nucleotide sequence encoding an amino acid sequence shown
20 by SEQ ID NO: 3.

7. The aldehyde oxidase gene according to claim 6 which has a nucleotide sequence shown by SEQ ID NO: 4 (loci of CDS being 91..4138).

8. A plasmid comprising the aldehyde oxidase gene
25 according to claim 1, 2, 3, 4, 5, 6 or 7.

9. A transformant transformed by introducing the plasmid according to claim 8 into a host cell.

10. The transformant according to claim 9, wherein the host cell is a microorganism.

5 11. The transformant according to claim 9, wherein the host cell is a plant.

12. A process for constructing an expression plasmid which comprises ligating (1) a promoter capable of functioning in a plant cell, (2) an aldehyde oxidase gene according to claim 1, 2, 3, 4, 5, 6 or 7 and (3) a terminator capable of functioning in a plant in a functional manner and in the said order described above.

13. An expression plasmid comprising (1) a promoter capable of functioning in a plant cell, (2) an aldehyde oxidase gene according to claim 1, 2, 3, 4, 5, 6 or 7 and (3) a terminator capable of functioning in a plant which are ligated in a functional manner and in the said order described above.

14. A process for controlling production of an aldehyde oxidase in a transformant which comprises introducing, into a host cell, an expression plasmid comprising (1) a promoter capable of functioning in a plant cell, (2) an aldehyde oxidase gene and (3) a terminator capable of functioning in a plant which are ligated in a functional manner and in the said order described above to transform said host cell.

15. The process according to claim 14, wherein the aldehyde oxidase gene is derived from a plant and the host cell is a plant cell.

16. The process according to claim 13, wherein the
5 expression plasmid is the expression plasmid according to
claim 13.

ABSTRACT

There is provided an aldehyde oxidase gene which is a
4.4 kbp gene obtainable from a plant and which encodes an
amino acid sequence of an enzyme capable of oxidizing an
5 aldehyde compound to a carboxylic acid and utilization
thereof.